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ORIGINAL ARTICLE

Chemical, mechanical and morphological properties of hypomineralized enamel of permanent first molars

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Abstract

Objective. The microstructure of hypomineralized enamel in permanent teeth has been described in several studies as less distinct prism sheaths and disorganized enamel with lack of organization of the enamel crystals. The mechanical properties, hardness and modulus of elasticity of the hypomineralized enamel have lower values compared with normal. The aim of this study was to examine normal and hypomineralized enamel using scanning electron microscopy (SEM), hardness measurements and X-ray microanalysis (XRMA). ***Material and methods.*** Four extracted hypomineralized permanent first molars, sectioned and cut in half, were analyzed with SEM, XRMA and hardness measurements. ***Results.*** An inverse relation was found between the micro hardness and the Ca:C ratio in hypomineralized and normal enamel. The acid-etched hypomineralized enamel appeared on SEM to be covered with a structureless layer and the prisms appeared disorganized, with thick prism sheaths and loosely packed crystallites. Furthermore, bacteria were found deep in porous hypomineralized enamel close to the enamel–dentin junction. ***Conclusions.*** Teeth diagnosed with molar incisor hypomineralization have significantly lower hardness values in the hypomineralized enamel compared with normal enamel. The hardness values vary according to the morphological and chemical properties.

Key Words: *Enamel, hardness, molar incisor hypomineralization, scanning electron microscopy, X-ray microanalysis*

Introduction

The condition molar incisor hypomineralization (MIH), defined as hypomineralization of the enamel in permanent first molars and incisors, has a reported prevalence varying between 2.8% and 37.5% [1]. There are a number of subjective and objective problems and complications associated with MIH, namely severe loss of enamel, hypersensitivity, dental fear, increased treatment need and problems in performing proper filling therapy [2–4]. Clinically, it is difficult to estimate the degree of hypomineralization and the risk of loss of enamel. However, it has been shown that there is a relation between hardness values and the color of the hypomineralized enamel, with yellow lesions being softer than white [5].

The microstructure of hypomineralized enamel of permanent teeth has been described in several studies as having less distinct prism sheaths and as disorganized enamel with a lack of organization of the enamel crystals [3,5–10]. The mechanical properties, hardness and modulus of elasticity of the hypomineralized enamel have lower values compared with normal enamel [5–10].

Elemental analyses of enamel in permanent first molars diagnosed with MIH have shown changes in the chemical composition and a reduction in the mineral composition [7,11,12]. Thus, a number of different methods have been used for studies of the morphological, chemical and mechanical properties of hypomineralized enamel in teeth with MIH.

The aim of this study was to examine normal and hypomineralized enamel utilizing scanning electron

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microscopy, hardness measurements and X-ray microanalysis. It was hypothesized that there is a higher content of organic matter, represented by an increase in carbon, in hypomineralized enamel and a lower hardness.

Material and methods

Tooth material

The tooth material consisted of four teeth from extracted permanent first molars with the diagnosis of MIH. After 24 h in 70% ethanol the teeth were embedded in an epoxy-resin (Epofix®; Electron Microscopy Sciences, Fort Washington, PA) and cut into two sagittal longitudinal parts in a Low Speed Saw Microtome® (Leitz, Wetzlar, Germany). The cutting was performed through the hypomineralized cusps.

The eight tooth halves were etched with 30% phosphoric acid, rinsed with deionized water, coated with gold by vapor deposition and analyzed using scanning electron microscopy (SEM).

Four tooth halves were prepared in the following way after the SEM analyses. The examined enamel surface was ground and polished, ≈0.5 mm was removed and hardness measurements were performed according to Vickers. All samples were examined in a stereo light microscope and photographs were taken.

SEM and X-ray microanalysis

The specimens were mounted on sample holders for the microscope and coated with gold by vapor deposition for SEM and carbon for X-ray microanalysis (XRMA). They were examined in a Philips SEM 515 scanning electron microscope (Philips, Eindhoven, The Netherlands) at 15 kV. For the elemental analyses, a Philips SEM 515, EDAX DX4, ECON detector was used. For all measurements, the X-rays were detected using a small window ($6.1 \times 4.3\ \mu m^2$). Additional SEM survey images were taken from the ground sections and the enamel surface and, immediately afterwards, an XRMA line scan was carried out. The amounts of the elements C, O, P and Ca were measured at the test points of the hardness measurements. The relative amounts of the measured elements were calculated with a computerized program (Point Electronic DISS 2; Point Electronic GmbH, Halle, Germany).

Hardness measurements

The four tooth halves examined using SEM were used for the hardness measurements and for second SEM/XRMA analyses. The cut surface of the specimens was polished prior to the hardness measurements. The final area of the specimen for the micro hardness measurements was ≈4 mm^2 (Figure 1). A digital micro hardness tester (Leitz Miniload 2; Wild Leitz, Wetzlar, Germany) fitted with a Vickers diamond and a 1.961-N load was used to make indentations in the polished enamel surface in normal and hypomineralized enamel. The loaded diamond was allowed to rest on the surface for 30 s. Three indentations were made in at least three locations within the normal and hypomineralized enamel. In the normal enamel, 45 indentations were made and in the hypomineralized enamel 54. Hardness was measured as Vickers pyramid numbers (HV).

Statistical analysis

The Mann–Whitney U-test of medians was used for statistical analyses to analyze differences between normal and hypomineralized enamel regarding the amounts of C, O, P, Ca and the ratios Ca:P and Ca:C registered in the XRMA analyses, and for the HV values.

Ethical considerations

All the examined teeth had been extracted due to severe hypomineralized enamel and for orthodontic reasons and were given freely by the patients. The teeth were stored in 70% ethanol in plastic tubes without any identification, so that none of the teeth could be traced to any specific patient.

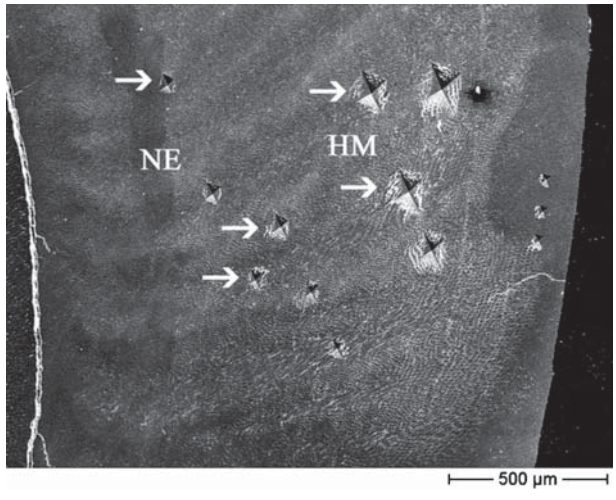

Figure 1. Light microscopic overview of locations for measurements of hardness and XRMA in enamel. NE = normal enamel; HM = hypomineralized enamel.

Results

In all examined teeth, the buccal and lingual surfaces, cusps and occlusal surfaces had porous, hypomineralized zones. The porous zones extended from the cuspal parts of the teeth comprising two-thirds of the buccal and/or lingual areas. The cervical parts of the enamel appeared normal. The border between the hypomineralized and normal enamel was distinct (Figure 2).

Hardness measurements

Values were significantly higher in normal enamel compared with hypomineralized enamel (Table I). Variations were larger in hypomineralized than normal enamel. In one sample, the HV value in hypomineralized enamel, close to the enamel surface, was the highest recorded.

XRMA results

The mean values for the three repeated XRMA measurements were calculated and used in the Mann–Whitney U-test. The median values for O, Ca and the Ca:P ratio did not differ between normal and hypomineralized enamel (Table II). The median value for C was statistically significantly higher in hypomineralized enamel compared with normal enamel, while P and the Ca:C ratio had significantly lower median values in hypomineralized compared with normal enamel (Table II).

The HV values and the values for Ca and C were normalized to respective values at the surface of normal enamel and the relative values for hardness, Ca and C were calculated and presented as relative percentages of the surface values for normal enamel (Figure 3). In normal enamel, the relative values for Ca, C and HV were thus 100% at the normal surface. However, the Ca values differed somewhat between normal and hypomineralized enamel at the surface and at the enamel–dentin junction (EDJ) the Ca values were higher for normal enamel. The C value had a flat gradient in normal enamel, whereas it increased from the enamel surface towards the EDJ in the hypomineralized enamel. As may be seen in Figure 3, the relative micro hardness values paralleled the relative carbon values in normal enamel; however, in hypomineralized enamel, the relative micro hardness values decreased from the enamel surface towards the EDJ, becoming almost parallel to the relative calcium values (Figure 3).

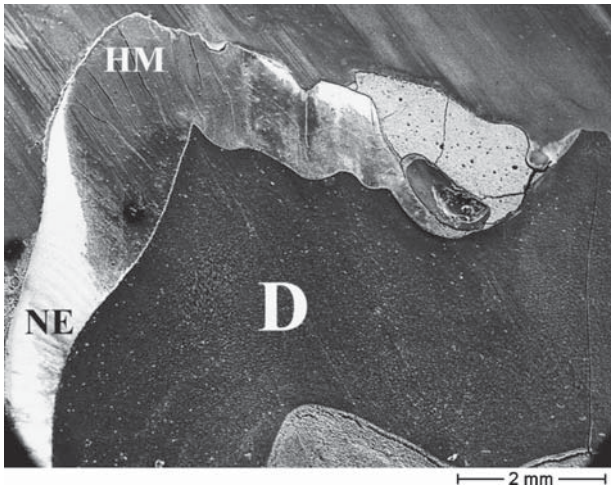

Figure 2. Low-magnification SEM image of a permanent first molar with normal and hypomineralized enamel. NE = normal enamel; HM = hypomineralized enamel; D = dentin.

Table I. Hardness (HV) measurements in normal and hypomineralized enamel.

	Normal	Hypomineralized
Mean	350.70	144.30
SD	30.15	106.54
Min.	284.00	15.00
Max.	411.00	437.00
Median	355.00	128.50*

*$W = 214.0$; $p = 2.06 \times 10^{-12}$.

SEM examination

At low magnification, the enamel appeared white and bright, while hypomineralized areas appeared dark (Figure 2). At higher magnifications, the normal enamel had a well-organized and distinct prism and crystal structure in contrast to the hypomineralized enamel, which had less distinct prism borders and crystals and more marked inter-prismatic space. Thus, the hypomineralized enamel appeared more porous than normal enamel (Figures 4a–4d). The enamel prisms in the hypomineralized enamel appeared to be covered by a structureless layer, in some locations appearing as less etched (Figures 5a–5b). The structureless layer appeared as a 'coating' covering the enamel. In some areas within the hypomineralized enamel, deep in a rupture of the structureless layer, enamel with non-covered irregular prisms could be seen (Figures 6a–6b). When an opening in the structureless layer was seen, several layers appeared between prisms oriented in the same plane (Figures 6a–6b). In other specimens, the layers were more evident, with several layers of prisms and organic matter and a porous region where bacteria could be seen (Figures 6c–6e).

In several specimens, bacteria were found on the enamel surface. In two specimens with a marked

Table II. XRMA measurements in normal and hypomineralized enamel.

	C (weight %)		O (weight %)		P (weight %)		Ca (weight %)		Ca:P (weight %)		Ca:C (weight %)	
	Norm	Hypo	Norm	Hypo	Norm	Hypo	Norm	Hypo	Norm	Hypo	Norm	Hypo
Mean	9.29	11.20	39.70	39.15	16.18	15.74	29.82	29.08	1.84	1.85	3.23	2.71
SD	0.65	2.33	0.56	1.02	0.28	0.59	0.83	1.66	0.03	0.06	0.30	0.58
Range	8.51–10.31	8.74–18.38	38.83–40.61	35.55–40.38	15.82–16.69	13.90–16.45	28.33–31.04	24.83–31.72	1.78–1.90	1.77–1.96	2.76–3.65	1.35–3.63
Median	9.29	10.62*	39.82	39.48	16.17	15.86*	30.01	29.56	1.84	1.84	3.26	2.76*

*$p < 0.01$.

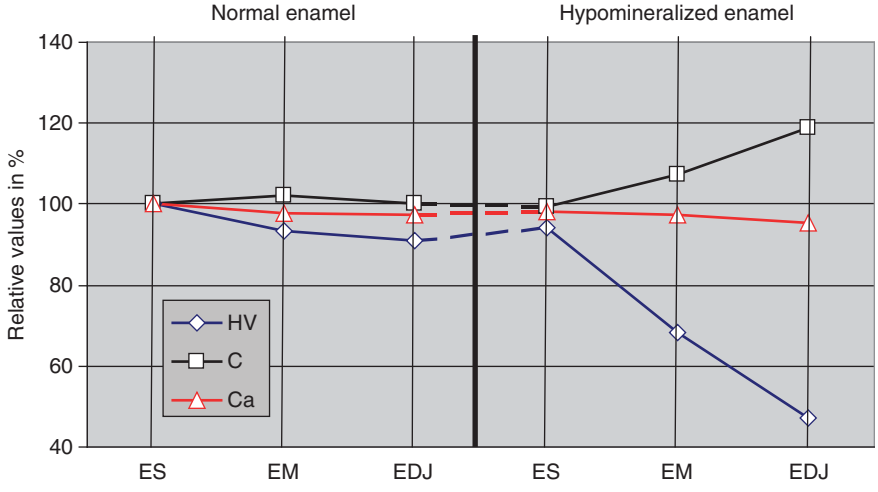

Figure 3. Graph showing the relative hardness and relative values for Ca and C in normal and hypomineralized enamel in three locations. ES = enamel surface; EM = middle of the enamel; EDJ = enamel–dentin junction.

inter-prismatic space, bacteria were found close to the EDJ (Figures 7a–7b).

Discussion

This study has shown that there is a relation between micro hardness and the Ca:C ratio in hypomineralized and normal enamel. The acid-etched hypomineralized enamel appeared to be covered by a structureless layer and the prisms appeared disorganized, with thick prism sheaths and loosely packed crystallites. Furthermore, bacteria were found not only at the enamel surface but also deep in hypomineralized enamel close to the EDJ.

The micro indentation technique has previously proven to be feasible for accurately determining both enamel and dentin, and therefore the method applied for obtaining hardness values may be regarded as suitable for the purpose of this study [12,13]. Furthermore, it is regarded that the results obtained using the micro indentation technique are not dependent on the prism orientation or on small variations in the chemical composition [14,15]. This is further strengthened by hardness measurements carried out across a caries lesion [16].

The findings of lower hardness values in hypomineralized compared with normal enamel coincide with what has been shown in other studies [5–7]. The high hardness value in one of the samples of hypomineralized enamel may be explained by its location close to the surface and, therefore, may be attributed to a post-eruptive mineralization. The Ca:P ratio did not differ significantly between normal and hypomineralized enamel, which is in agreement with several other studies [7]. This is in contrast to a previous study by Jälevik et al. [11]; however, other studies have reported a stable Ca:P ratio despite differences in the degree of mineralization [17]. The conflicting results in the literature have been discussed by Mahoney et al. [7], leaving the reasons open for further discussion. However, one of the main problems with the Ca:P ratio is what it actually represents. In hypomineralized enamel with a higher content of organic matter, P observed in the XRMA analyses may possibly therefore derive not only from the hydroxyl apatite but also from the organic matter.

The higher relative concentration of C in the hypomineralized enamel may explain the SEM appearance of the hypomineralized enamel after acid etching, with a structureless layer covering the enamel prisms. This is supported by findings in a previous paper, where

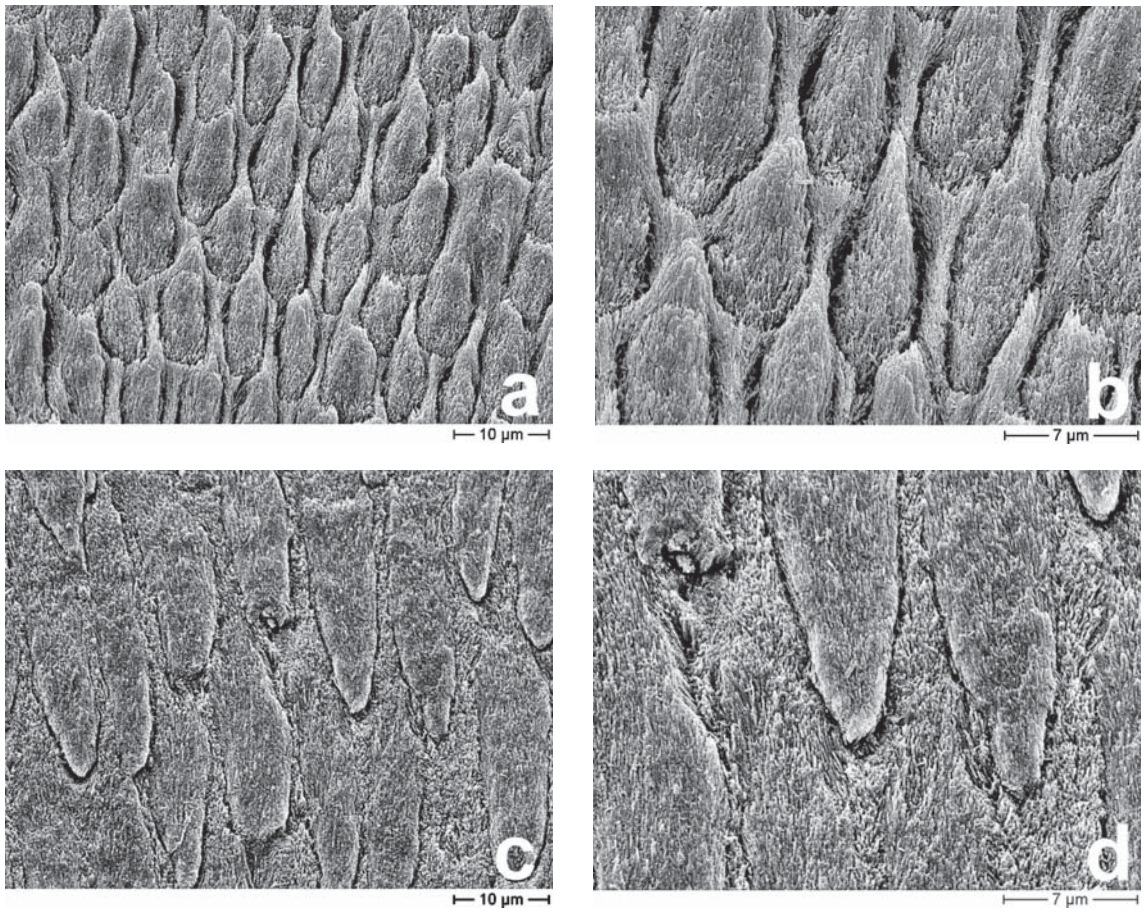

Figure 4. SEM images of normal and hypomineralized enamel of a permanent first molar. (a) Normal enamel with well-organized, distinct prisms (original magnification ×2000). (b) The same area as in (a) but at higher magnification (original magnification ×4000). (c) Hypomineralized enamel showing less distinct enamel prisms (original magnification ×2000). (d) The same area as in (a) but at higher magnification (original magnification ×4000).

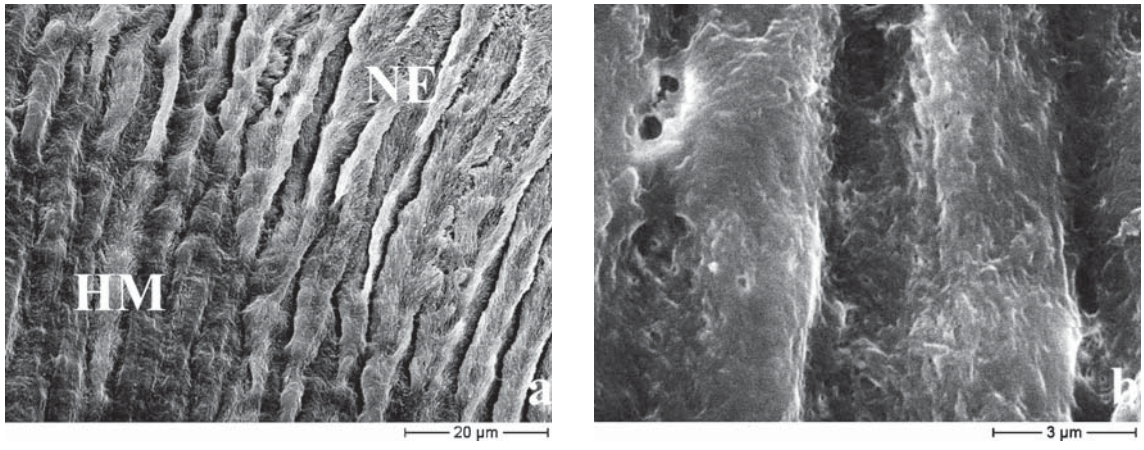

Figure 5. SEM images of hypomineralized enamel from a permanent first molar. (a) Interface between normal and hypomineralized enamel from a permanent first molar with dark hypomineralized enamel and bright normal enamel. NE = normal enamel; HM = hypomineralized enamel (original magnification ×1500). (b) Enamel prisms in hypomineralized enamel covered by a structureless layer (original magnification ×10 000).

thicker prism sheaths and a higher intra- as well as inter-prismatic organic content were found [9].

The morphological and chemical appearance of a well-demarcated hypomineralization within the tissue with a distinct border towards the normal enamel could suggest an early insult to the ameloblasts from which they apparently do not recover. However, a prerequisite for completion of mineralization is that the enamel matrix is removed [18] and, in animal experiments, it has been shown that dioxin causes

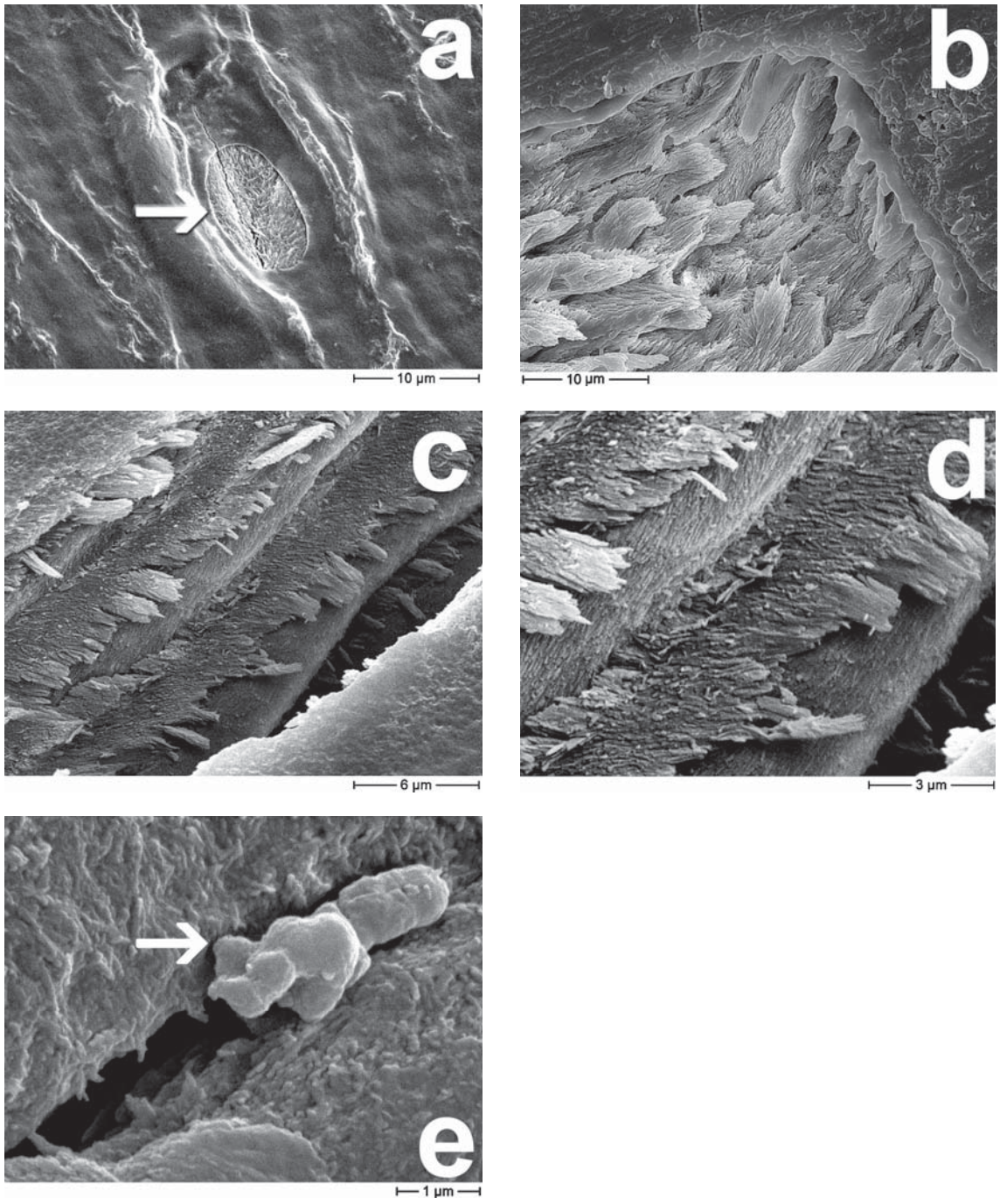

Figure 6. SEM images of hypomineralized enamel of a permanent first molar. (a) Rupture of the structureless layer showing underlying prisms and crystallites (original magnification ×3000). (b) Rupture of the structureless layer showing underlying enamel prisms and another structureless layer (original magnification ×3000). (c) Hypomineralized enamel in the middle part of the enamel showing porous enamel with sheets of organic material between layers of disorganized prisms (original magnification ×5000). (d) Higher-magnification image of the same area (original magnification ×10 000). (e) Higher-magnification image from the same area as in (c) and (d) showing bacteria (original magnification ×20 000).

retention of enamel matrix and is likely to be an early sign of disturbed function of secretory dental cells [19]. Morphological studies of hypomineralized enamel of rat pups fed on a calcium-deficient diet have shown that the enamel was hypomineralized and that the crystallites were thinner than in normal enamel. Furthermore, organic matrix was found in the enamel [20]. In the latter study, calcification was restored when the rats returned to a normal diet. Diet-induced chronic hypocalcaemia in rats interfered with cellular and extracellular events during enamel maturation [21]. It is not unlikely that the ameloblasts forming the hypomineralized enamel in MIH are affected by a hypocalcaemic state and by another agent. In the case of MIH, the calcification is evidently not restored, which would indicate that the

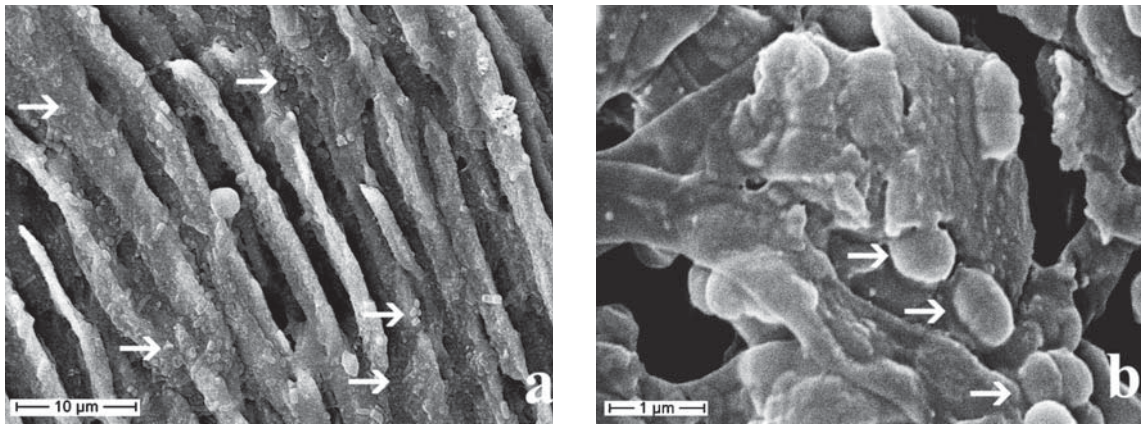

Figure 7. SEM images of hypomineralized enamel from a permanent first molar. (a) Bacteria found in the enamel close to the EDJ on the surface of the enamel prisms (magnification 2500×). (b) Bacteria found in the enamel close to the EDJ on the surface of the enamel prisms (magnification 20 000×).

maturation stage is irreversibly affected. Nevertheless, the exact etiology and mechanism of MIH are still not known.

The finding of a structureless layer with underlying prisms may be explained by the fact that the acid etching affects the porous hypomineralized enamel more than normal enamel, thus exposing the organic part of the prism sheaths. This may be attributed to the less inorganic content of the hypomineralized enamel. The disorganized enamel prism, porous structure and loosely packed crystallites seen at higher magnifications confirm the results of other investigators [6,7,9–19].

The findings of bacteria in the hypomineralized enamel close to the EDJ may be explained by the extremely porous enamel seen in the SEM images. This explains the hypersensitivity of hypomineralized permanent first molars, which is further supported by the finding of bacterial penetration through the hypomineralized enamel into the dentin in the cuspal areas of permanent first molars shown in a previous article [22].

Porous enamel with disorganized prisms and wide prism sheaths creates substantial problems, not least concerning survival of fillings and teeth [4]. When the hypomineralized enamel is etched, the filling then 'faces' an organic layer rather than enamel with a normal etch pattern and thus the filling will not function as desired. Since restorative material does not entirely eliminate micro leakage [23] and possible bacterial penetration through surrounding hypomineralized enamel, the clinical outcome may be problematic for both the patient and clinician.

Conclusions

Teeth diagnosed with MIH have significantly lower hardness values (HV) in hypomineralized compared with normal enamel. This authenticates the post-eruptive breakdown of MIH teeth, which is one of many clinical objective symptoms of MIH. Bacteria were observed deep in the enamel, adjacent to the EDJ. This confirms earlier findings that bacteria may penetrate to the deeper part of the tooth and are a probable cause of the hypersensitivity seen in MIH teeth.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Willmott NS, Bryan RA, Duggal MS. Molar-Incisor-Hypomineralisation: A literature review. Eur Arch Paediatr Dent 2008;9:172–9.

[2] Leppäniemi A, Lukinmaa PL, Alaluusua S. Nonfluoride hypomineralizations in the permanent first molars and their impact on the treatment need. Caries Res 2001;35: 36–40.

[3] Jälevik B, Klingberg GA. Dental treatment, dental fear and behaviour management problems in children with severe enamel hypomineralization of their permanent first molars. Int J Paediatr Dent 2002;12:24–32.

[4] Mejàre I, Bergman E, Grindefjord M. Hypomineralized molars and incisors of unknown origin: treatment outcome at age 18 years. Int J Paediatr Dent 2005;15:20–8.

[5] Suckling GW, Nelson DG, Patel MJ. Macroscopic and scanning electron microscopic appearance and hardness values of developmental defects in human permanent tooth enamel. Adv Dent Res 1989;3:219–33.

[6] Mahoney E, Ismail FS, Kilpatrick N, Swain M. Mechanical properties across hypomineralized/hypoplastic enamel of first permanent molar teeth. Eur J Oral Sci 2004;112: 497–502.

[7] Mahoney EK, Rohanizadeh R, Ismail FS, Kilpatrick NM, Swain MV. Mechanical properties and microstructure of hypomineralised enamel of permanent teeth. Biomaterials 2004;25:5091–100.

[8] Jälevik B, Dietz W, Norén JG. Scanning electron micrograph analysis of hypomineralized enamel in permanent first molars. Int J Paediatr Dent 2005;15:233–40.

[9] Xie Z, Kilpatrick NM, Swain MV, Munroe PR, Hoffman M. Transmission electron microscope characterisation of

molar-incisor-hypomineralisation. J Mater Sci Mater Med 2008;19:3187–92.

[10] Xie ZH, Mahoney EK, Kilpatrick NM, Swain MV, Hoffman M. On the structure-property relationship of sound and hypomineralized enamel. Acta Biomater 2007;3: 865–72.

[11] Jälevik B, Odelius H, Dietz W, Norén J. Secondary ion mass spectrometry and X-ray microanalysis of hypomineralized enamel in human permanent first molars. Arch Oral Biol 2001;46:239–47.

[12] Fearne J, Anderson P, Davis GR. 3D X-ray microscopic study of the extent of variations in enamel density in first permanent molars with idiopathic enamel hypomineralisation. Br Dent J 2004;196:634–8; discussion 625.

[13] Cuy JL, Mann AB, Livi KJ, Teaford MF, Weihs TP. Nanoindentation mapping of the mechanical properties of human molar tooth enamel. Arch Oral Biol 2002;47: 281–91.

[14] Braly A, Darnell LA, Mann AB, Teaford MF, Weihs TP. The effect of prism orientation on the indentation testing of human molar enamel. Arch Oral Biol 2007;52:856–60.

[15] Kodaka T, Debari K, Yamada M, Kuroiwa M. Correlation between microhardness and mineral content in sound human enamel (short communication). Caries Res 1992; 26:139–41.

[16] Angker L, Swain MV, Kilpatrick N. Characterising the micro-mechanical behaviour of the carious dentine of primary teeth using nano-indentation. J Biomech 2005;38: 1535–42.

[17] Kodaka T, Debari K, Kuroiwa M. Mineral content of the innermost enamel in erupted human teeth. J Electron Microsc 1991;40:19–23.

[18] DenBesten PK, Heffernan LM, Treadwell BW, Awbrey BJ. The presence and possible functions of the matrix metalloproteinase collagenase activator protein in developing enamel matrix. Biochem J 1989;264:917–20.

[19] Gao Y, Sahlberg C, Kiukkonen A, Alaluusua S, Pohjanvirta R, Tuomisto J, et al. Lactational exposure of Han/Wistar rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin interferes with enamel maturation and retards dentin mineralization. J Dent Res 2004;83:139–44.

[20] Bonucci E, Lozupone E, Silvestrini G, Favia A, Mocetti P. Morphological studies of hypomineralized enamel of rat pups on calcium-deficient diet, and of its changes after return to normal diet. Anat Rec 1994;239:379–95.

[21] Nanci A, Mocetti P, Sakamoto Y, Kunikata M, Lozupone E, Bonucci E. Morphological and immunocytochemical analyses on the effects of diet-induced hypocalcemia on enamel maturation in the rat incisor. J Histochem Cytochem 2000;48:1043–58.

[22] Fagrell TG, Lingström P, Olsson S, Steiniger F, Norén JG. Bacterial invasion of dentinal tubules beneath apparently intact but hypomineralized enamel in molar teeth with molar incisor hypomineralization. Int J Paediatr Dent 2008;18: 333–40.

[23] Zicković S. Bojović S, Palica D. Bacterial penetration of restored cavities. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91:353–8.